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OCCURRENCE AND BIOREMEDIATION OF ANTHRACENE IN THE ENVIRONMENT

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ABSTRACT

Occurrence of PAH in the environment has been a concern of many environmentalists for its obstinac, toxicity and harm that it may impose. Anthracene is a common low molecular weight PAH that is often used as a model PAH in bioremediation study due to its structure that is also found in high molecular weight PAH. This article provides an overview of the occurrence of PAH and specifically, anthracene. Due to the harm that they impose, several methods have been applied to overcome the problem, including opting for bioremediation. This article will explore on the bioremediation process involving microorganisms and the respective enzymes secreted during the process. Proposed mechanism on degradation of anthracene by bacteria and fungus are also included. The paper will further leads to gaps of study and to the direction for future study and applications.

Keywords: anthracene; bacteria; fungus; microorganisms; polycyclic aromatic hydrocarbon (PAH).

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1. INTRODUCTION

PAHs are polycyclic aromatic hydrocarbons having at least two fused benzene ring arranged in a linear, angular or clustered manner [1]. There are 16 PAHs listed by USEPA [2]. Their physical characteristics include low solubility in water, high melting and boiling points and low vapor pressure. Generally, the higher the molecular weight of a PAH, its melting and boiling point increase while vapor pressure and solubility in water, decrease[3]. PAHs are also toxigenic, genotoxic, mutagenic and carcinogenic [4]. Due to these characteristics, the release of polycyclic aromatic hydrocarbons (PAHs) into air, soil, water and marine environment concerns environmentalists for the harm PAHs impose.

PAHs are introduced into the environment through various ways that branch into anthropogenic and natural sources [5]. Its presence in the environment however is exacerbated by anthropogenic activities [6]. PAHs may undergo various fates in the environment that include volatilization, photo-oxidation, chemical oxidation, adsorption by soil particles, leaching and microbial degradation [7-8]. Physical and chemical methods are also applied for effective removal of PAHs. Bioremediation, an alternative to conventional methods has been extensively explored for its potential to remove PAHs from the environment [9].

Anthracene, as seen in Fig. 1 is a three-ring linear PAH isomeric with phenanthrene which is arranged in an angular manner.

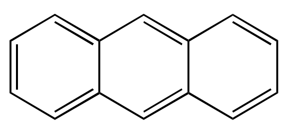

Fig.1. Anthracene structure

Although it is a low molecular weight PAH listed by USEPA as priority pollutant, it is not acutely toxic, carcinogenic or mutagenic [6, 10]. Anthracene however, attacks the skin, stomach, intestines and the lymphatic system upon entering the body system [11]. Anthracene is often chosen as model PAH in various bioremediation studies because its structure is also found in benzo(a)anthracene and benzo(a)pyrene[12].

2. OCCURRENCE OF ANTHRACENE IN THE ENVIRONMENT

Major source of PAHs entering the environment is from the incomplete combustion of organic materials [13]. The increase of anthropogenic activities has also led to the widespread of PAH infiltrating the environment [14]. Industrial sites are usually associated with high PAH contamination due to activities such as processing, combustion and disposal of fossil fuels with regards to spills and leaks from storage tanks [15]. Anthracene and its methyl derivatives are common pollutants in cigarette smoke, coal liquefaction product, diesel exhaust and shale oils [16]. It is also found in soil, natural water, sediments, sewage or wastewater treatment plant, in the atmosphere and even in food [17]. According to [18], more than 95% of anthracene can be found in water while less than 1.4% can be found in atmosphere. When anthracene is released in soil, it will not leach into ground water but binds to soil particles instead.

3. REMOVAL AND BIODEGRADABILITY OF PAH AND ANTHRACENE

Removal, alteration or isolation of pollutants through excavation, incineration or containment have been applied for PAH removal. However, these methods are expensive and often involve transferring PAH from one phase to another. Alternatively, biodegradation offers an inexpensive and practical solution to the problem by transforming PAH to its less or non-toxic metabolites. The method requires much less input of chemicals and energy [19].

PAH degrading organisms span across both plant and animal kingdom that often involves microbes that are mostly bacteria, algae or fungi. Biodegradation occurs by breaking down of organic compounds through biotransformation into less complex metabolites or through mineralization into water, carbon dioxide or methane [7].

Fig. 2 shows the proposed pathway of biodegradation of anthracene[12]. Biodegradation of anthracene involved two major pathways with various metabolites in between. Continuous reaction resulted either 9,10-anthraquinone, 1-methoxy-2-hydroxyathracene, 3-(-2-carboxyvinyl)naphthalene-2-carboxylic acid or 6-7-benzo(a)coumarin which finally proceeded to ring fission process.

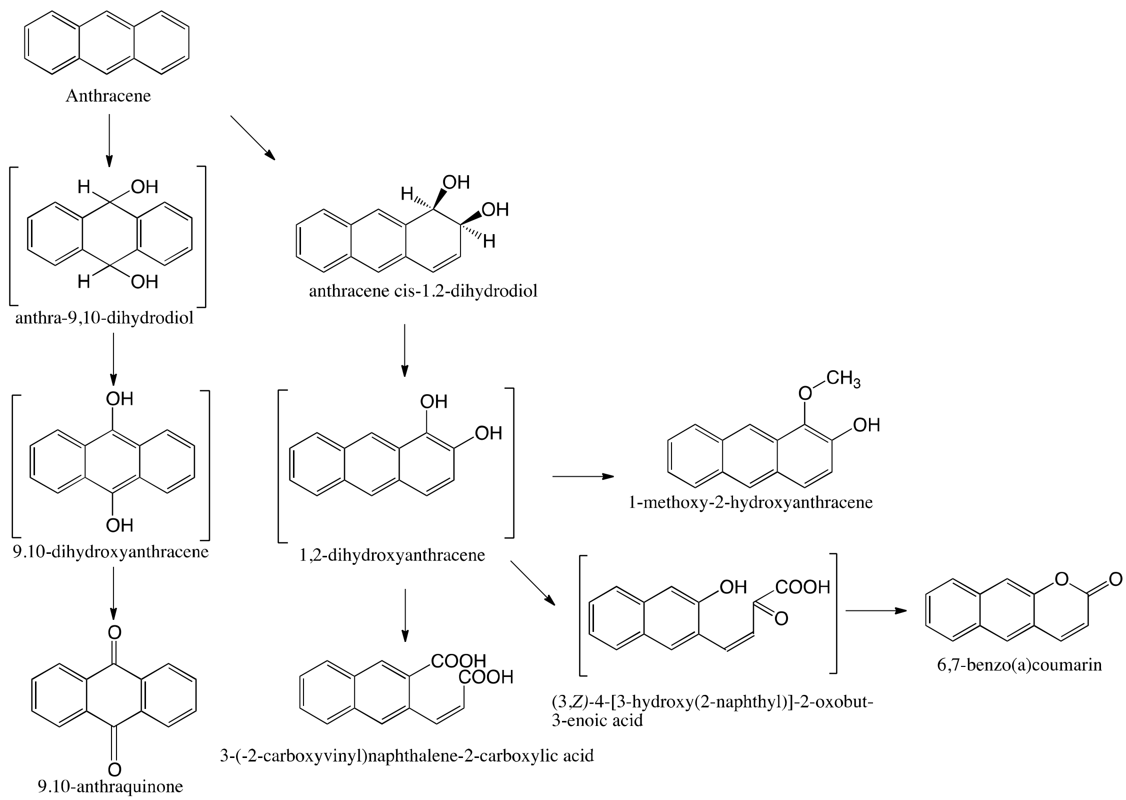

Fig.2. Proposed pathway of anthracene biodegradation [12]

Anthracene undergoes various fates when it is released into the environment. When it is found in natural water, photodegradation occurs. This process is influenced by numerous factors such as intensity of solar radiation, time of the day, latitude of the site, depth and turbidity of water bodies. When water turbidity is high, the rate of photodegradation is slower due to light scattering and the low intensity of sunlight reaching the pollutant. Anthracene trapped in sediment from adsorption on the other hand, would not photodegrade as a result of insufficient light [20]. Anthracene had shorter half-life with high exposure of sunlight[17].

Very limited evidence is available for phytoremediation of anthracene in soil environment, as compared to degradation by bacteria or fungi. Both bacteria and fungi have been widely explored for their xenobioticsdegradation including PAHs. However, due to different adaptability, factors including pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of compounds or cellular transport properties are to be considered to determine the rate of biodegradation of an organism [7].

Table 1 shows the common parameters considered for bioremediation study and respective effects on biodegradation activity. Table 2 shows the maximum effect of parameters on biodegradation performance. The parameters involved posed a significant impact on biodegradation of pollutant [9, 21-23]. The optimum conditionsfor biodegradation process result in maximum pollutant degradation.

Table 1. Parameters considered for biodegradation study

Parameters	Effects
Temperature	Degradation velocity
Water content	Pollutant and degraded product transport
pH	Excretion of extracellular enzymes crucial for bioremediation activity
Redox potential	Determines pathway and efficiency of degradation
Solubility, Sorption, Volatility, Occlusion	Relates to bioavailability of pollutants to the reach microorganisms
Nutrient	Improve growth and reproduction of microbes
Co-contaminant	Enhance or inhibit bioremediation
Microbial communities	Determines the bioremediation rate
Co-substrate	Enable co-metabolic transformation of contaminant

Table 2. Parameters effect on biodegradation by different organism

Parameters	Types of Organisms	Maximum Effect of Bioremediation
Concentration of oil	Bacteria Nostochatei Pseudoalteromonas sp., Reugeria sp., Exiguobacterium sp., Acinetobacter sp. Fungus Penicillium commune Candida tropicalis	Maximum at low concentration of oil in the media.
Temperature	Bacteria Nostochatei	Max at 28°C

	Pseudoalteromonas sp., Reugeria sp., Exiguobacterium sp., Acinetobacter sp.	Max at 30 °C
	Fungus	
	Penicillium commune	Max at 27 °C
	Candida tropicalis	Max at 30 °C
Degradation period	Bacteria	
	Nostochatei	7 days for 2% (w/w) waste lubricant
	Pseudoalteromonas sp., Reugeria sp., Exiguobacterium sp., Acinetobacter sp.	30 days for 0.5-4.0% (v/v) waste motor oil
	Fungus	
	Penicillium commune	15 days for 1% (v/v) spent engine oil
	Candida tropicalis	10 days for 1-75 μL of industrial oil 3 days for 5.3% of hydrocarbon consumption
pH	Bacteria	
	Nostochatei	Max at pH 7.5
	Pseudoalteromonas sp., Reugeria sp., Exiguobacterium sp., Acinetobacter sp.	Max at pH 8.0
	Fungus	
	Penicillium commune	Max at pH 5.5
	Candida tropicalis	Max at pH 8.0

3.1. Bioremediation of Anthracene by Bacteria

Bacteria are a class of microbes well-established in the field of bioremediation. Most of the involved bacteria are isolated from the contaminated site. Long-term exposure of bacteria to the contaminants enables them to tackle to a considerable extent [24]. Several bacterial strains have been reported in degrading PAH including Rhodococcus sp., Alteromonas sp., Cycloclasticus, Arthrobacter, Bacillus, Mycobacterium sp. and Pseudomonas sp. [25-26]. Bacillus, Escherichia and Mycobacterium are common bacterial strains that have been reported to degrade not only anthracene and several other PAHs in the presence of heavy metals, but lighten the burden brought by heavy metals [24].

The two key steps in bacterial degradation of PAH are initial oxidative attack followed by cleavage of benzene ring [27]. The oxidative attack leads to formation of diol, ring cleavage and eventually dicarboxylic acid. Common bacterial strains found in marine environment are Pseudomonas, Acinetobacter, Nocardia, Vibro and Achromobacter[28]. In [7] reported that a 98% reduction of total PAH content was achieved in 6 months by mixed microbial culture of bacteria from genera Acenitobacter and Klebsiella. Another study of [29] on the other hand reported Rhodococcus sp. isolated from sediments of River Grand Calumet had initial reaction rates of $0.47 \mu g L^{-1}$ for anthracene mineralization. In another study, a group of bacteria successfully degraded 57.1% of naphthalene, 82.1% of phenanthrene and 55.2% of anthracene[30]. Meanwhile, in[12] also reported that Mycobacterium sp. strain PYR-1 exposed to anthracene successfully degraded 92% of the pollutant after 14 days of incubation. 3-(2-carboxyvinyl)naphthalene-2-carboxylic acid and 6,7-benzocoumarin were detected as anthracene's metabolites after degradation process.

These studies are examples that bacterial strains are capable of degrading and using pollutants as an alternative solution to conventional PAH removal methods.

3.2. Bioremediation of Anthracene by Fungi

Just like bacteria, fungiare microorganisms that degrade PAH. Lignolytic fungi produce extracellular enzymesthat areinduced by irregular structure of lignin. For this reason, the lignolytic system by this strain has low substrate specificity and hence capable of degrading different types of compounds. Enzymes associated with the system are lignin peroxidase, manganese dependent peroxidase, laccase and hydrogen peroxide-producing enzyme [7]. Fungi present a great potential for bioremediation because of its biomass production and excessive growth. An advantage that puts fungi ahead of bacteria is that fungi do not require preconditioning to the pollutant [31]. Isolated Aspergillusfumigatus from contaminated site degraded 65% of anthracene when the condition was set at pH 5.0 to 7.5 and temperature at

30◦C [32].The study also proposed a pathway for anthracene degradation, which resulted into phthalic acid, as shown in Fig. 3 [32].

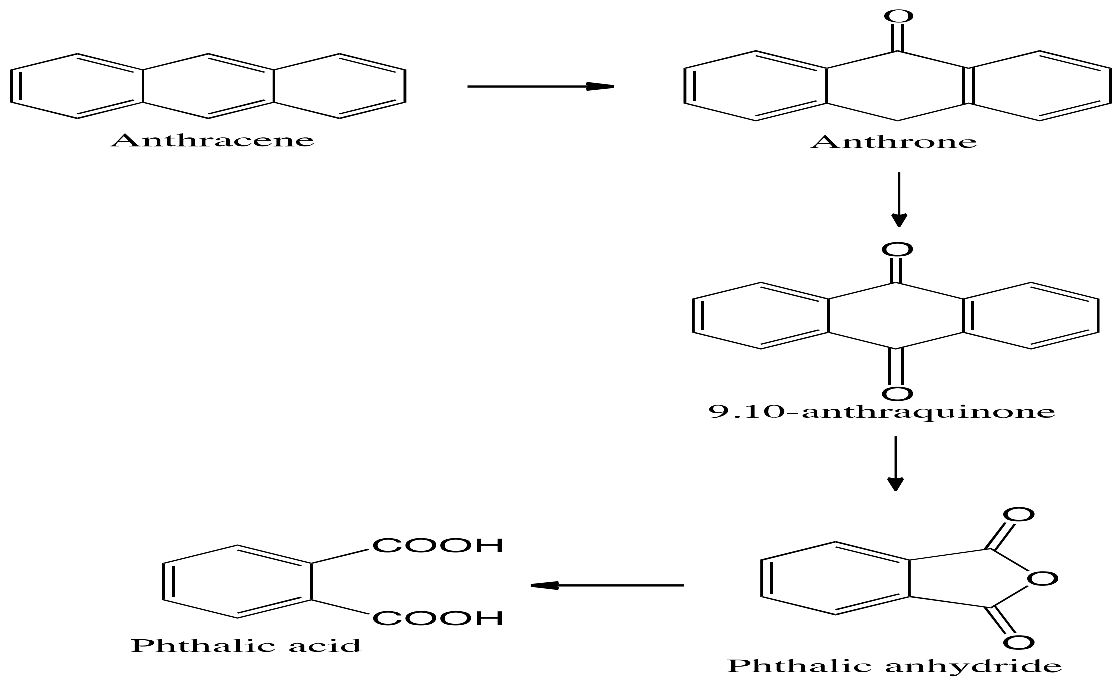

Fig.3. Proposed anthracene degradation pathway [32]

In [33] reported that strains from genera Bjerkandera, Phanerochaete, Ramaria andAgaricales were capable of transforming anthracene to anthraquinone but as a dead-end metabolite. In another study, a total of 40 strains isolated from an artificial wetland with most of the strains degraded 0.01 g L^{-1}anthracene at different rates under liquid cultivation conditions. Ulocladiumchartarum and Absidiacylindrospora were the most efficient strains by degrading more than 80% during treatment period [34]. White rot fungi for example, employs lignin degradation system for bioremediation process [7]. Many PAHs possess ‘Bay region’ and ’K region’, which are sites for formation of bay and K-region epoxides. These epoxides are highly reactive [35]. Table 3 shows the degradation of anthracene by various fungal strains, the metabolites resulted from the degradation and the enzymes involved in the process [34, 36-37]. Enzymes as indicated in Table 3 are fundamental in degradation of PAH process when fungi are involved. Enzymes play a role by bindingto PAH at a specific region, before the metabolites are further degraded by ring fission process. From the studies mentioned previously, it is evident that fungal strains are useful to overcome the increasing abundance of PAH in the environment.

Table 3. Fungal strains and enzymes involved in degradation resulting in anthracene metabolites

Fungal Strain	Enzymes	PAH Metabolites
Phanerochaetechrysosporium	Lignin peroxidase (LiP)	9,10-Anthraquinone
Anthracophyllum discolor	Mn-dependent peroxidase (MnP)	Not determined
Strophariacorronila	Mn-dependent peroxidase (MnP)	9,10-Anthraquinone
Pleurotusostreatus	Laccase (LAC)	9,10-Anthraquinone
Trametesversicolor	Laccase (LAC)	9,10-Anthraquinone
Bjerkanda genera, Phanerochaete genera, Ramaria genera, Agaricales genera	Mn-dependent peroxidase (MnP)	9,10-Anthraquinone
Aspergillusfumigatus	Lignin peroxidase (LiP)	Phthalic anhydride, anthrone and anthraquinone
Ulocladiumchartarum	Cytochrome P-450 monooxygenase	Not stated
Absidiacylindrospora	Cytochrome P-450 monooxygenase	Not stated

4. CONCLUSION

Opting for solutions to overcome the increasing amount of PAHs in the environment is essential for sustainability of the world. An economical, non-environmental destructive method must be employed instead of the current conventional methods that are expensive and produce secondary pollutant. Anthracene is proven to be biodegradable and various potential microorganisms can be utilized to achieve biodegradation objective. The outcome of these studies will give an understanding on how biodegradation of anthracene commences and further contributes on potential for future studies.

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